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Photosystem II and the evolution of the light-harvesting antennae in vascular plants: a new concept.

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Abstract
Cryo-electron crystallography of grana membranes has led to a new concept of the positioning of photosystem II (PSII) relative to its peripheral light-harvesting complex (LHCII). In projection, structural data reveals small domains surrounding the central PSII core which are compatible with the size and expected stoichiometry of the LHCII proteins. When viewed in 3D, however, these small domains are shown to occupy a membrane separate from the membrane plane that houses the PSII core region. This observation fits with the known morphology of the grana membrane preparation that consists of paired, tightly appressed membranes. The structural data has been confirmed by separate biochemical experiments where LHCII-enriched and core PSII-enriched membrane fractions have been isolated.

Introduction
The nature of the organisation of the PSII/LHCII complex in the grana membranes of higher plants has been the subject of several recent studies using electron microscopy. There is a lack of consensus between these various studies, with a tetrameric and occasionally dimeric association of PSII/LHCII observed for detergent-solubilised material (Boekema et al., 1995; Hankamer et al., 1997; Nicholson et al., 1996), whilst studies of the complex *in-situ* reveal a monomeric organisation for some workers (Holzenburg et al., 1993; Ford et al., 1995) whilst others interpret the data as showing a dimeric association (Marr et al., 1996). The case for a dimeric organisation has been strengthened by the recent X-ray crystallography studies of cyanobacterial PSII (Zouni et al., 2001), although arguments concerning detergent-solubilisation as a driver of artificial oligomerisation hold also for the cyanobacterial system, and it is debatable how far one can extrapolate from a prokaryotic system *in-crystallo*. Clearly, cautious interpretation is desirable, especially when extrapolating to the organisation of a eukaryotic photosystem *in-vivo*. The more important question of the nature of the interaction of PSII core with its LHCII antennae subunits has also lacked consensus. Studies have assigned a LHCII trimer at each end of a dimeric complex (Boekema et al., 1995), based on the observation of a roughly triangular density in the projection maps. In contrast, studies of PSII/LHCII *in-situ* have assigned monomeric LHCII subunits to small peripheral domains that encircle a central core region (Holzenburg et al., 1993).

In this paper, we use the X-ray crystallography structure of cyanobacterial PSII core to demonstrate the monomeric nature of PSII in higher plants. More significantly, we show 3D data that suggests a radical revision of our ideas concerning the organisation of the LHCII and PSII core complexes. This data suggests that LHCII occupies a separate membrane to the PSII core complex.

Materials and methods

Barley *viridis* zb63 grana membranes were prepared, and electron microscopy was performed as described in (Stoylova et al., 1997 & 2000). Image processing was carried out with the software developed at the MRC-LMB Cambridge (Amos et al.,1982). Table I lists the number of files employed in the different tilt ranges, demonstrating that reciprocal space is oversampled by the data with low phase residuals. A 3D Coulomb density map for the cyanobacterial PSII core complex was calculated using the SPIDER image processing software package (Health Research Inc. New York) and inputting the PDB file *1fe1 (*Zouni et al., 2001*)*. This file lacks the extramembraneous loops of the transmembrane protein subunits and one of the extrinsic subunits of the cyanobacterial PSII core complex, which remain to be identified in the electron density map. The resolution was artificially curtailed at 30Å resolution using Fourier filtration. Isolation of membrane fractions from grana membranes was carried out by treatment with Tris base (1.5M Tris-hydroxymethyl aminomethane, pH8.8) for 2 hr in subdued light at 20^{o}C, followed by freeze-thaw overnight, and then the membranes collected by centrifugation for 2hr at 110,000 xg in a Beckman SW41 rotor onto a cushion composed of 2M sucrose in buffer A (20mM morpholinoethanesulfonate (MES), 5mM $MgCl_2$, 15mM NaCl, pH 6.3). The sharp green band at the interface was collected and frozen at –20^{o}C; thawed and loaded onto a linear gradient composed of 0 to 2M sucrose in 0.75M Tris-base, 3M urea, pH 8.8. After centrifugation for 2hr as above, green bands corresponding to different membrane fractions were harvested. Membranes were diluted 1:1 with distilled water and then centrifuged as above to obtain pellets. The membranes were analysed by absorbance spectroscopy, SDS-PAGE and electron microscopy as described in (Stoylova et al., 1997 & 2000).

Table I: Summary of the 3D data for the PSII/LHCII crystals.

Scan step at the specimen level:	6.6 Å or 8.9 Å	
No. of crystalline areas	168	
Maximum tilt angle:	±66^{0}	
No of files in tilt range:	0-30^{o}	69
	30-40^{o}	14
	40-50^{o}	28
	50-60^{o}	54
	60-66^{o}	2
No. of observations (to 30Å)	5066	
No. of structure factors used	470	
Overall weighted phase residual to 30 Å (where 90^{0} is random)	24^{0}	

Results

Figure 1 compares the 3D structure of higher plant PSII/LHCII *in-situ* with the 3D structure of one monomer of the cyanobacterial PSII core complex *in-crystallo* (Zouni et al., 2001), with both structures displayed at the same scale and resolution. It is clear that a close similarity in terms of shape and size applies, with the cyanobacterial monomer only slightly smaller than the higher plant core. A distinctive cavity on the lumenal side of the higher plant complex bounded by four domains (I-IV) has been previously described (Holzenburg et al., 1993; Ford et al, 1995). Domains I and II were assigned to extrinsic proteins 33kDa and 23kDa (Ford et al., 1995; Holzenburg et al., 1996) in the higher plant complex. The location of the 33kDa protein (PsbO) is confirmed, whilst the 23kDa protein is apparently replaced by cytochrome c550 in the prokaryotic complex. A distinctive feature of the higher plant structure is the presence of several small densities that occupy a separate plane in 3D space to the core domain. These appear to interconnect between core domains in the crystalline lattice. A plausible interpretation of this is that the small densities occupy a separate and distinctive

membrane to the one housing the core domain. This observation is consistent with the fact that the crystals are found in paired BBY membranes. Disruption of the grana membrane pairs by chaotrope treatment, followed by density gradient centrifugation allowed the isolation of two membrane fractions of radically different pigment and protein composition. Whilst one membrane fraction was enriched in core PSII polypeptides and chlorophyll *a*, the other was enriched in LHCII and chlorophyll *b* (data not shown).

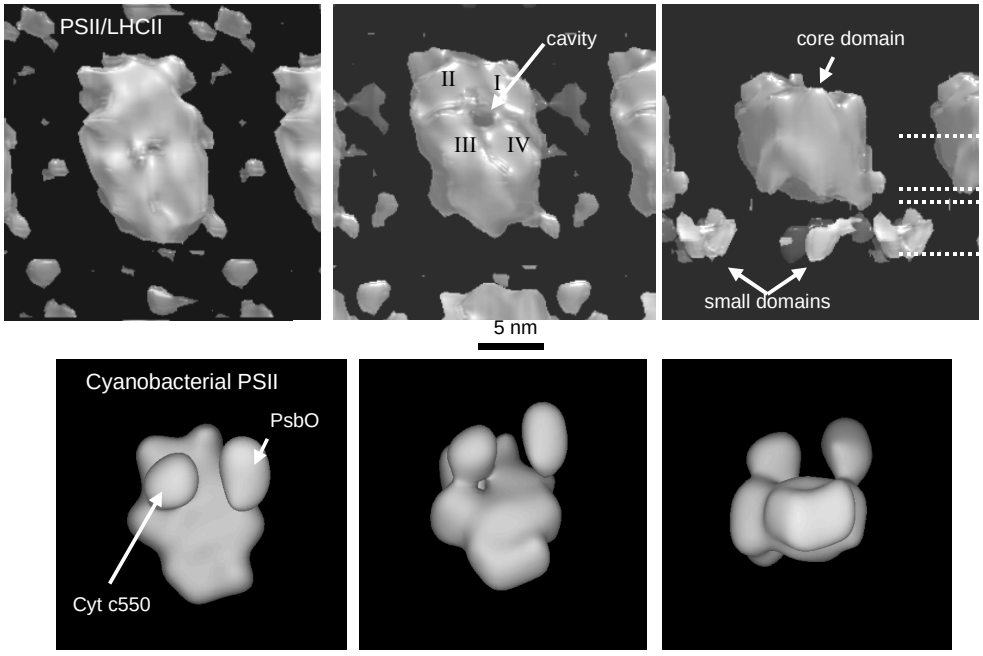

Fig. 1 Higher plant PSII/LHCII (top row) and cyanobacterial PSII (bottom row) shown at the same scale with 3 views rotated around a horizontal axis. Face-on view from the lumenal side (left), end-on view along the membrane (right), and an intermediate rotation (centre). The dashed lines (top right) indicate the putative boundaries of 2 membranes.

Discussion

The high resolution structure of a cyanobacterial PSII core complex has allowed a direct comparison with eukaryotic PSII studied *in-situ*. The data displayed in Figure 1 clearly show that higher plant PSII is consistent with a cyanobacterial PSII monomer. This therefore implies that tetrameric and dimeric higher plant complexes observed after detergent solubilisation are not an accurate reflection of the *in-vivo* state. A vertical segregation of LHCII and PSII into separate membranes within the grana of higher plants is proposed on the basis ofy evidence from structural and biochemical data. Such a segregation could be of wide significance: (a) Optimisation of light harvesting capacity (packing one membrane with LHCII, whilst at the same time maintaining efficient diffusion of plastoquinone in an adjacent membrane loosely packed with PSII core complexes). (b) Rapid adaptation to changes in light quality and intensity (via physical separation/appression of membrane pairs). (c) Cooperativity (PSII core complexes can tap into a large LHCII antenna located in an adjacent membrane). (d) Insights into possible routes for the evolution of light harvesting in plants (cyanobacteria move light excitation energy vertically from the phycobilisome to PSII core via linker proteins). Figure 2 presents a new model for PSII/LHCII organisation in higher plants and attempts to

draw an evolutionary link with cyanobacterial and green sulphur bacteria where light excitation energy is also moved perpendicular to the membrane plane.

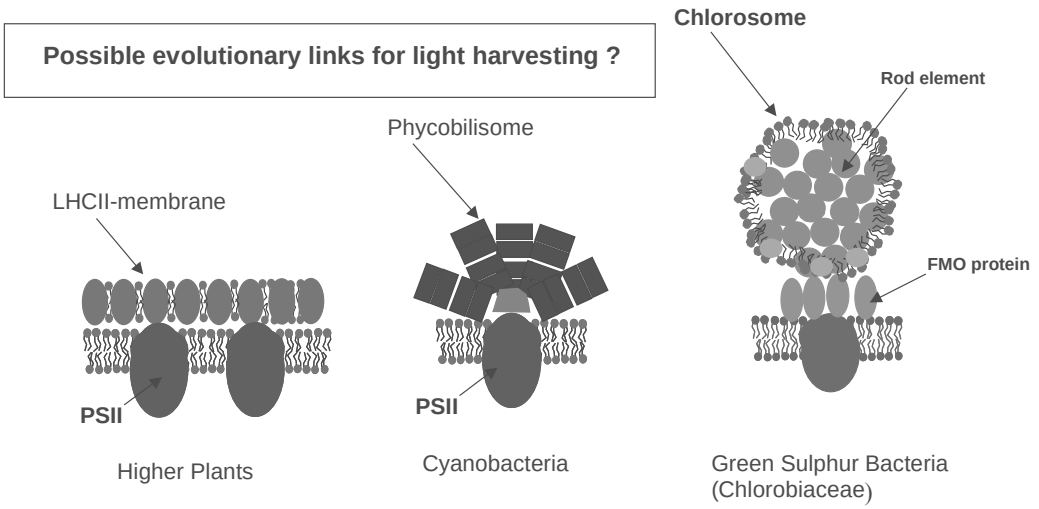

Fig. 2 Model of an appressed grana membrane pair containing a LHCII-enriched and a core PSII-enriched membrane (left). Movement of light excitation energy in a direction perpendicular to the membrane also occurs in cyanobacteria (centre) and green sulphur bacteria (right).

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